

APPENDIX B

PENDING AND AMENDED CLAIMS

1. (Once amended) An isolated nucleic acid that encodes a fusion polypeptide, wherein the fusion polypeptide comprises:

a) a catalytic domain of a glycosyltransferase that catalyzes the transfer of a saccharide, from a saccharide donor comprising a nucleotide sugar, to an acceptor molecule; and

b) a catalytic domain of an accessory enzyme that catalyzes the formation of the nucleotide sugar.

2. (As filed) The nucleic acid of claim 1, wherein the glycosyltransferase is a eukaryotic glycosyltransferase.

3. (As filed) The nucleic acid of claim 1, wherein the accessory enzyme is a eukaryotic accessory enzyme.

5. (As filed) The nucleic acid of claim 1, wherein the glycosyltransferase is a prokaryotic glycosyltransferase.

6. (As filed) The nucleic acid of claim 1, wherein the accessory enzyme is a prokaryotic accessory enzyme.

7. (As filed) The nucleic acid of claim 1, wherein the fusion polypeptide further comprises a catalytic domain of a second accessory enzyme.

8. (As filed) The nucleic acid of claim 1, wherein the glycosyltransferase is selected from the group consisting of sialyltransferases, N-

acetylglucosaminyltransferases, N-acetylgalactosaminyltransferases, fucosyltransferases, galactosyltransferases, glucosyltransferases, glucuronosyltransferases, xylosyltransferases, and mannosyltransferases.

9. (Once amended) The nucleic acid of claim 1, wherein the accessory enzyme is selected from the group consisting of:

- a GDP-mannose dehydratase;
- a GDP-mannose 3,5-epimerase;
- a GDP-mannose 4-reductase;
- a UDP-glucose 4' epimerase;
- a UDP-GalNAc 4' epimerase;
- a CMP-sialic acid synthetase;
- a neuraminic acid aldolase;
- an N-acetylglucosamine 2' epimerase;
- a phosphate kinase selected from the group consisting of a pyruvate kinase, a myokinase, a creatine phosphate kinase, an acetyl phosphate kinase, and a polyphosphate kinase; and

- a pyrophosphorylase selected from the group consisting of a UDP-Glc pyrophosphorylase, a UDP-Gal pyrophosphorylase, a UDP-GalNAc pyrophosphorylase, a GDP-mannose pyrophosphorylase, a GDP-fucose pyrophosphorylase, and a UDP-GlcNAc pyrophosphorylase.

10. (As filed) The nucleic acid of claim 1, wherein the nucleotide sugar is selected from the group consisting of GDP-Man, UDP-Glc, UDP-Gal, UDP-GlcNAc, UDP-GalNAc, CMP-sialic acid, GDP-Fuc, and UDP-xylose.

11. (As filed) The nucleic acid of claim 1, wherein the glycosyltransferase is a sialyltransferase and the nucleotide sugar is CMP-sialic acid.

12. (As filed) The nucleic acid of claim 11, wherein the accessory enzyme is a CMP-sialic acid synthetase.

23. (Once amended) The nucleic acid of claim 1, wherein the catalytic domain of the glycosyltransferase and the catalytic domain of the accessory enzyme are joined by a peptide linker.

24. (As filed) The nucleic acid of claim 1, wherein the nucleic acid further comprises a polynucleotide that encodes a signal sequence which is linked to the fusion polypeptide

25. (As filed) The nucleic acid of claim 1, wherein the nucleic acid further comprises a polynucleotide that encodes a molecular tag which is linked to the fusion polypeptide.

26. (Once amended) An expression vector which comprises the nucleic acid of claim 1.

27. (Once amended) A host cell which comprises the expression vector of claim 26.

33. (Once amended) A method of producing a fusion polypeptide, the method comprising:

- a) introducing into a host cell the expression vector of claim 26, under conditions where the host cell is transformed with the expression vector; and
- b) culturing the transformed host cell under conditions where the fusion polypeptide is expressed in the transformed host cell.

34. (Once amended) The method of claim 33 further comprising a step of purifying the expressed fusion polypeptide.

35. (Once amended) The method of claim 33 further comprising a step of permeabilizing the host cell expressing the fusion polypeptide.

37. (New) An isolated nucleic acid that encodes a fusion polypeptide, wherein the fusion polypeptide comprises:

a) an α -2,3-sialyltransferase that catalyzes the transfer of a sialic acid, from CMP-Neu5Ac, to an acceptor molecule; and

b) a CMP-Neu5Ac synthetase that catalyzes the formation of CMP-Neu5Ac from Neu5Ac and CTP.

38. (New) The nucleic acid of claim 37, wherein the α -2,3-sialyltransferase and the CMP-Neu5Ac synthetase are joined by a peptide linker.

39. (New) The nucleic acid of claim 37, wherein the nucleic acid further comprises a polynucleotide that encodes a signal sequence which is linked to the fusion polypeptide

40. (New) The nucleic acid of claim 37, wherein the nucleic acid further comprises a polynucleotide that encodes a molecular tag which is linked to the fusion polypeptide.

41. (New) The nucleic acid of claim 37, wherein the α -2,3-sialyltransferase is a bacterial enzyme.

42. (New) The nucleic acid of claim 41, wherein the α -2,3-sialyltransferase is a *Neisseria* enzyme.

43. (New) The nucleic acid of claim 37, wherein the CMP-Neu5Ac synthetase is a *Neisseria* enzyme.

44. (New) An expression vector which comprises the nucleic acid of claim 37.

45. (New) A host cell which comprises the expression vector of claim 45.

46. (New) A method of producing a fusion polypeptide, the method comprising:

a) introducing into a host cell the expression vector of claim 45, under conditions where the host cell is transformed with the expression vector; and

b) culturing the transformed host cell under conditions where the fusion polypeptide is expressed in the transformed host cell.

47. (New) The method of claim 47 further comprising a step of purifying the expressed fusion polypeptide.

48. (New) The method of claim 47 further comprising a step of permeabilizing the host cell expressing the fusion polypeptide.